An Immunological Study Including the Relationship of Interleukin 12, 17, TNF, TGF, and Women Infected with Toxoplasmosis

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Abstracts: Toxoplasmosis, a condition that is known to produce several physiological changes in infected women, is brought on by the intracellular parasite Toxoplasma gondii. This study aimed to determine a relationship between latent toxoplasmosis and the concentrations of IL-12, IL-7, TNF, and TGF-β. A total of 250 samples from infertile women were gathered, and about 24% of the samples revealed toxoplasma gondii infection. Both qualitative and quantitative anti-Toxoplasma IgG levels were measured utilizing an immunofluorescent assay carried out on automated VIDAS family devices. The amounts of IL-12, IL-17, TNF, and TGF-β were also evaluated using the ELISA method, which uses an enzyme-linked immunosorbent assay.

Keywords: *Toxoplasma gondii,* IL-12, IL-17, TNF, TGF-β.

1. INTRODUCTION

The potential to infect a sizable section of the world's population of humans exists due to the widespread distribution of the protozoan parasite “Toxoplasma gondii”. In terms of zoonotic and veterinary health, it is also capable of infecting a variety of warm-blooded animals, making it a significant pathogen. Due to the parasite's significance in both medicine and veterinary science, there has been a lot of research into it. Additionally, due to its unicellular structure, it is a perfect model organism for research on molecules and cell biology (1). A large percentage of the world's population could potentially become infected with the parasite Toxoplasma gondii, which is a widespread protozoan. As a result of its capacity to infect a variety of warm-blooded species, it is a significant disease in terms of zoonotic and veterinary health. The parasite's significance in medicine and veterinary science has attracted a lot of attention in the field of research. Additionally, because it is a single cell, it is a perfect model organism for research into molecular mechanisms and cell biology. Consuming raw or preserved animal meats that have tissue cysts with T. gondii is another method of transmission (4). Severe clinical signs of toxoplasma infection, including myocarditis, meningoencephalitis, retinochoroiditis, and can develop and may be incurable. Most infected individuals, however, exhibit few or no pathological symptoms and are thus symptomless. As a result, latent Toxoplasmosis infection was long thought to have a mild influence on public health, apart from immunocompromised patients (5). It's interesting to gain insight into how toxoplasma parasite and nervous system disorders are related. The severity of toxoplasmosis' effects on humans, who are unintentional intermediate hosts, appears to rely on the health of their immune systems (6). The immune system is an intricate network of organs and cells that collaborates to keep the body in a state of homeostasis and respond to dangers from the outside (35). In the first defense against infection, the innate immune system is essential. Neutrophils, monocytes, and dendritic cells (DCs) all produce interleukin-12 (IL-12) when exposed to an infection. However, after phagocytosing Tg, macrophages do not respond to infection by producing IL-12 (1, 7).

It is important to note that the type of cell and the strain of Tg responsible for the infection both affect the human cellular response to Tg infection (8). While IFN-γ is the primary cytokine in charge of preventing Tg infection, other cytokines have also been linked to the process. For instance, by generating interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNFα), brain microglial cells help regulate Tg growth. TNFα is thought to play a role in mediating Tg death in patients with IFNγ receptor 1 (IFNGR1) loss, partially making up for the absence of IFNγ responsiveness (7). A crucial cytokine produced by T helper 17 (Th17) cells, IL-17 is important for both the immune response to infections and autoimmune disorders.

The cytokine IL-17 is referred to as either IL-17A specifically or the entire IL-17 family, which includes IL-17A, IL-17C, IL-17F, IL-17B, IL-17D, and IL-17E (also known as IL-25). The most in-depth research has been done on IL-17A, the IL-17 family member that was first identified. A careful balance between the parasite's evasion strategies and the host's immune system determines the persistence of Toxoplasma gondii, an intracellular parasite, in

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different intermediate hosts. It's interesting to note that different infected species and cell types produce different immune reactions. Contrarily, IL-17, a major factor in the immunopathological reactions to toxoplasmosis, regulates Th17 cells, which play a significant role in generating inflammation. As a biomarker for the severity of the toxoplasmosis condition, IL-17 has also been discovered. An immunoregulatory cytokine called TGF-β is involved in the development of Th17 cells and the regulation of Th1/Th2-driven immune responses (11). During different infections, lymphoid cells such as Th17, Tc17, γδT cells, and ILC3 are the main producers of IL-17A. Several infections' induction of neutrophils also helps to produce IL-17A (12). While the first innate immune response, which is predominantly driven by neutrophils, is crucial for successfully resolving the infection, the cell-mediated response is as important for defending against Toxoplasma gondii. Neutrophil depletion investigations have shown that the infection gets worse when they are lost. However, little research has been done on the variables affecting how the neutrophil response to infection develops. Interleukin 17 (IL-17), according to recent research, may have a substantial role in neutrophil growth and recruitment (13). For healthy fertilization and pregnancy, the decidual immune system, whether adaptive or innate, must exist. This system includes many T helper cell subtypes (Th1, Th17, Th2, T follicular helper, CD8+ T, CD28+, and regulatory T-cells), as well as autoantibodies such as antiphospholipid antibodies, antithyroid antibodies, and antiovarian antibodies.

Moreover, cytokines, chemokines, and other elements are involved. Infertility and failed pregnancies can result from changes in the ratios or concentrations of these adaptive immune system components (14). The polypeptide protein known as tumor necrosis factor (TNF) is first made as a pro-peptide. TNF-α converting enzyme (TACE) is the enzyme that transforms it into the active form, TNF-alpha. TNF-alpha, which has 157 amino acids, is mostly created when macrophages, dendritic cells, and T lymphocytes are activated. T. gondii infection decreases the TNF-alpha production of B lymphocytes (15). TNF is a cytokine that can cause both pro- and anti-inflammation.

It is crucial for the emergence of cancer, autoimmune disorders, and defense against pathogenic organisms. TNF can operate on T cells and be produced by T cells, among its many other functions (16). TNF-alpha is a cytokine that promotes inflammation and has a variety of impacts on different cell types. It is fundamental in the emergence of chronic illnesses. TNF-alpha is produced by both immune and non-immune cells, including astrocytes, granuloma cells, fibroblasts, glial cells, and keratinocytes (17). Immune cells that produce TNF-alpha include B cells, natural killer cells, T cells, basophils, eosinophils, dendritic cells, neutrophils, and mast cells. More than 30 members of the Transforming Growth Factor β (TGF-β) superfamily are secreted by platelets.

The immune system, tissue healing, and extracellular matrix synthesis are all impacted by TGF-β1 (34). The suppression of the proliferation and operation of many immune system components is an important aspect of maintaining immunological homeostasis and tolerance (18). The three TGF-β isoforms (TGF-β1, -β2, and -β3) are essential for controlling gene expression, cell motility, and differentiation. They have been linked to both reparative and fibrotic reactions, with evidence for their role in tissue fibrosis coming from cell biology research, animal models, and clinical observations (19). When three or more consecutive pregnancies end spontaneously before the 20th week of gestation, this is referred to as recurrent spontaneous abortion (RSA).

Although several reasons, including infections, genetic abnormalities, anatomical problems, immunological variables, and endocrine diseases have been linked to RSA (20), the exact etiology is still unknown in between 40 and 60 percent of individuals. Worldwide, there is a high prevalence of toxoplasma infection, and 20–90% of adults have serum toxoplasma antibodies. The presence of cats in homes, age, place of residence, cleanliness, dietary habits, gender, and cultural variables all have an impact on where this parasite is found. The parasites multiply inside host cells and can spread throughout the body via the bloodstream, infecting different cell types. When a fetus has congenital toxoplasmosis, the infection can spread through the placenta, potentially causing catastrophic harm (21).

2. MATERIALS AND METHODS

2.1. Subjects

2.1.1. Control

50 healthy samples were gathered from Hila City for the control group. The subjects were asked a series of questions by an interviewer to rule out any people who had any medical conditions.

2.1.2. Patient

A total of 250 subjects were collected, and all cases were questioned about their reproductive capacity, number of pregnancies, and the incidence of any chronic illnesses.

2.2. Blood Collection

Participants gave five milliliters of blood, which was then transferred to a gel tube. The blood was centrifuged at 50 RPM for 10 minutes to separate the serum from the entire blood. Once chilled to -20 degrees Celsius, the blood was kept in a refrigerator until it was required.

2.3. Immuno-Fluorescent Assay

Using an enzyme-linked fluorescence immunoassay (ELFA), anti-toxoplasma IgG can be found in serum. This technique combines a final fluorescence detection with an immunocapture enzyme immunoassay. Using a commercially available kit made by the French company Biomerieux, the test method is carried out.

2.4. Measure the IL-12, IL-17

Enzyme-Linked Immunosorbent Assay (ELISA) was used to test the levels of IL-12 and IL-17 using an ELISA kit *from Monobind Inc. in the United States.*

2.5. Statistical Analysis

SPSS 10.0 (SPSS Inc., Chicago, USA) was used to conduct the statistical analyses. An ANOVA test was used to investigate categorical variables. The average and standard deviation of the results were displayed. Comparative statistics between the patient group and the healthy group were run. Statistically significant differences were considered to exist at a significance level of p 0.05.

3. RESULT AND DISCUSSION

The age range of 65% of the patients—250 patients and 50 controls—is 20 to 29 years. Patients who are between the ages of 30 and 39 make up a smaller fraction, according to Table 1.

Table 1. Distribution of the patients according to the age groups.

Patients	**20-29 years**		**30-39 years**		**Controls**
Infected with Toxoplasma gondii	**33(53%)**		**29(47%)**		**50**
	25 IgM Positive	**8 IgG Positive**	**15 IgM Positive**	**14 IgG Positive**	
Infected without Toxoplasma Gondi	**69 (39%)**		**109 (61%)**		
Totally	**112**		**138**		**50**

According to a study, the prevalence of a specific illness was evaluated about age, and their tables demonstrated that the infection that occurs is high in the aged group (20-29 years). The study found a non-significant trend toward an age-related increase in prevalence ($P > 0.05$). Following are the prevalence rates for various age groups:

- 5.2–5.3% of people are under 20 years old.
- People 20 to 29 years old: 7.0%
- People between the ages of 30-39: 6.0%
- People 40 years of age or older: 4.8 to 9.7%

According to the study, the age group 40–49 had the highest prevalence of the illness, at 9.7%. Additionally, it showed a pattern where the rate of positivity was lowest in those under the age of 20, and it increased steadily with age, reaching its highest level in those between the ages of 40 and 49. This age group had a seropositive rate that was around twice as high as those under the age of 20. According to this result, compared to other age groups, those between the ages of 20 and 29 have a higher percentage of toxoplasmosis infections. By detecting antibodies in serum samples, serological assays are useful for identifying Toxoplasma gondii infection and determining whether the infection is acute or chronic. The prevalence of the T. gondii parasite in women varies throughout nations as a result of conditions like climate, dietary habits, socioeconomic status, degree of education, and age. 69 serum samples from a group of 250 women who had abortions were discovered to contain anti-Toxoplasma antibodies. In the 69 blood samples from the 250 women who had abortions, 25 of these positive results were identified as anti-IgM antibodies and 8 as anti-IgG antibodies. The latter group, which includes those between the ages of 20 and 29, had a seropositive rate of 9.7%.

This rate was roughly twice as high as the seropositive rate seen in the under-20 age group. This data reveals that compared to other age groups, the 20–29 age group had a larger percentage of toxoplasmosis infections. Toxoplasma gondii infection can be accurately diagnosed by serological techniques. To assess whether the infection is acute or persistent, these approaches involve looking for antibodies in serum samples. The prevalence of the T. gondii parasite in women might vary depending on several variables, including climate, dietary habits, socioeconomic situation, education level, and age. These elements may vary depending on the nation. 69 serum samples from the 250 women who had abortions had anti-Toxoplasma antibody results that were positive.

In the 69 blood samples from the 250 women who had abortions, out of these positive results, 25 were identified as anti-IgM antibodies, indicating acute infection, and 8 were identified as anti-IgG antibodies, indicating chronic

infection. The current study is in line with a prior study (reference 23), which found that women in Baghdad who had spontaneous recurrent miscarriages had a seroprevalence of 59% for toxoplasma IgG antibodies and 8% for toxoplasma IgM antibodies. According to the study, patients between the ages of 20 and 29 had the highest percentage of infected patients (53%) while patients between the ages of 30 and 39 had the lowest percentage (47%) of infected patients.

3.1. Interleukin -17, Transforming Growth Factor–β and Tumors necrosis factor cytokines detection

Enzyme-Linked Immunosorbent Assay (ELISA) was used to quantify the amounts of IL-17 and TGF-β cytokines. An equation for a typical curve fit was used to determine the findings. According to the current investigation, there was a significantly significant rise in the average level of IL-17 in patient serum (250.114 pg/ml) as compared to the control group (213.772 pg/ml). This result is in line with a 2012 study by Mohammed et al. that also found that patients with IgM seropositivity had significantly higher serum IL-17 levels. In the Mohammed et al. trial, IL-17 blood levels were 54.875 pg/ml in the control group, 54.182 pg/ml in the post-treatment group, and 266.589 pg/ml in the IgM seropositive group.

The early rise in serum IL-17 levels seen in the current study is consistent with the findings of earlier studies (24) that also noted an early rise in IL-17 during the early stages of infection. Neutrophils are essential for removing parasites in the early stages of infection, and IL-17 is known to have a role in their growth and early recruitment. Low IL-17 levels may impair the body's capacity to eliminate parasites. Before this study, it was thought that TGF-β mainly served as an immunoregulatory cytokine, directly inhibiting T-cell activation while indirectly promoting immunological responses that promote tolerance or suppressive responses.

However, new research has shown a previously unrecognized function of TGF-β, which collaborates with IL-6 and other inflammatory cytokines to promote the development of a specific subgroup of T-helper cells known as Th17 cells (25). In this investigation, the mean serum TGF-β, concentration in the patient group was 56.305 pg/ml, compared to 24.935 pg/ml in the control group. The difference between the two groups was statistically significant, which is consistent with a previous study's (26) finding that infection-related changes in PDL-1, IL-10, and TGF-β levels were also statistically significant. According to the findings, PDL-1, IL-10, and TGF-β levels might be used as biomarkers for Toxoplasma gondii's detrimental effects on developing fetuses.

When mice were infected with toxoplasmosis, there was a reduction in placental TGF-β levels that was linked to poor pregnancy outcomes (27). In a different study, pregnant women with acute T. gondii infection had higher TGF-β levels than pregnant women without the infection (26). Additionally, pregnant women with anti-Toxoplasma antibodies had greater TGF-β levels, and these levels were associated with typical pregnancy outcomes. In our research, we discovered that toxoplasmosis patients had greater levels of TNF-α than the control group did. This result deviates from a prior study (28) which claimed that TNF-α levels remained constant throughout toxoplasmosis.

Our results contradict a different study's (29) assertion that T. gondii inhibits the generation of TNF-α. TNF-α is an inflammatory and immune response cytokine that, together with IL-6, aids in promoting B lymphocyte expansion and subset specialization (29). Additionally, it triggers the release of acute-phase proteins by IL-6 production and initiates the killing of protozoa by eosinophils. TNF-α and IFN-γ also have anti-proliferative qualities. TNF-α appears essential for activating macrophages and preventing parasite proliferation in toxoplasmosis. However, achieving this result requires collaboration with IFN-γ.

3.2. Correlation between IL17 and TGF beta

The correlation between TGF-β and IL-17 systemically was positive and the p-value was significant at less than 0.05 p-value, Figure (2)

3.3. Correlation between IL17 and TNF

In our investigation, we found an overall positive association between IL-17 and TNF, but the p-value was not statistically significant at 0.05 as shown in Figure (3). Other studies revealed a relative association between interleukins and TNF (39).

3.4. Correlation between TNF and TGF

In our investigation, a systemic positive connection between TNF and TGF was identified; nevertheless, the p-value was not statistically significant at 0.05, as shown in Figure 5. The present analysis confirms earlier findings (30, 37) that infection rates were highest in those aged 26–35 and lowest in those aged 35–45. Frequent interaction with cats or tainted produce may be to blame for the increased seropositivity rates in the 26–35 age range. This result is in line with the findings of a different Iranian study (31) that also found a significant frequency of seropositivity in the 25–30 age group. The current study also found that the mean level of IL-17 in patient serum was significantly higher than in the control group (250.114 pg/ml) compared to 213.772 pg/ml.

The increase was very noteworthy. These results are consistent with those of the study (31), which showed that patients with IgM seropositivity had significantly higher serum levels of IL-17 (266.589 pg/ml) than did the control and post-treatment groups (54.875 pg/ml and 54.182 pg/ml, respectively). The results of several researchers (32) who reported a comparable early increase in IL-17 during the early stages of infection are consistent with the early elevation of IL-17 serum levels seen in the current investigation. These researchers discovered that IL-17 contributes to neutrophil growth and recruitment, which are essential for getting rid of parasites in the early stages of infection. Furthermore, it was discovered that the same cytokine, IL-12, enhanced IgG synthesis while not inhibiting IgE at low concentrations. Several cytokines, including IFN-γ, IL-2, and IL-12, are essential in the body's defense against parasite infections. IFN-γ and TNF production is sparked by IL-12, and lymphocyte-mediated cytotoxicity is also activated (33, 36). Furthermore, the immune response to parasite infections is thought to be mediated by both IFN-γ and IL-10.

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